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SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: Patrick Lewis Examiner #: 79002 Date: 7-22-03
Art Unit: 1623 Phone Number 30 5-4043 Serial Number: 09/485,071
Mail Box and Bldg/Room Location: CM1/8D12 Results Format Preferred (circle): PAPER DISK E-MAIL
CM1/8-B-19

If more than one search is submitted, please prioritize searches in order of need.

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Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: Modulators of DNA Cytosine-5-Methyltransferase and Methods ~~for~~ for Use Thereof

Inventors (please provide full names): Norbert O. Reich ; James Flynn

Earliest Priority Filing Date: 8-29-1997

For Sequence Searches Only Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.

* Bib Data Sheet attached

A synthetic oligonucleotide of at least 26 nucleotides in length and comprising a 5mCpG dinucleotide, wherein the 5mC is a C-5 methylcytosine, and which comprises a nucleotide sequence ~~selected~~ SEQ ID NO: 10

CTGGATCCTT~~G~~GCCCCGCCCC
TTGAATTCCC

Please Rush

PAlutzell

peg 10 NA 30

STAFF USE ONLY

Searcher: D. Schreiber
Searcher Phone #: 308-4292
Searcher Location: CM1 6A03
Date Searcher Picked Up:
Date Completed: 7/24
Searcher Prep & Review Time: 15
Clerical Prep Time:
Online Time: 46

Type of Search

NA Sequence (#)
AA Sequence (#)
Structure (#)
Bibliographic ✓
Litigation
Fulltext
Patent Family
Other

Vendors and cost where applicable

STN 108.50
Dialog
Questel/Orbit
Dr. Link
Lexis/Nexis
Sequence Systems
WWW/Internet
Other (specify)

PTO-1590 (1-01)

Lewis 09/485,071

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=> d his

     (FILE 'HOME' ENTERED AT 17:49:08 ON 24 JUL 2003)

     FILE 'REGISTRY' ENTERED AT 17:49:18 ON 24 JUL 2003
L1             5 S CTGGATCCTTGCCCCGCCCCTTGAAT|TGGATCCTTGCCCCGCCCCTTGAATT|GGATCCT
                 E CYTOSINE/CN
                 E CYTOSINE, 5
                 E CYTOSINE, 5/CN
L2            13 S CYTOSINE, 5-METHYL?/CN

     FILE 'HCAPLUS' ENTERED AT 18:00:08 ON 24 JUL 2003
L3           128 S REICH N?/AU
L4           579 S FLYNN J?/AU
L5           699 S L3 OR L4
L6             3 S L1
L7          1537 S L2
L8           218 S 5(A)METHYL(3A)CYTOSINE
L9             3 S L5 AND L6
L10            0 S L6 AND (L7 OR L8)
L11            3 S L6 AND (METHYLTRANSFERASE# OR METHYL(A)TRANSFERASE#)
L12          338 S ?MCPG
L13            0 S L12 AND L6
L14          266 S METHYL(3A)CPG
L15            0 S L14 AND L6
L16          635 S METHYL?(3A)DINUCLEOTIDE#
L17            1 S L16 AND L6
L18           62 S L16 AND (METHYLTRANSFERASE# OR METHYL(A)TRANSFERASE#)
L19           42 S L16 (L) (METHYLTRANSFERASE# OR METHYL(A)TRANSFERASE#)
L20            1 S L19 AND L6
L21            3 S L6 OR L9 OR L11 OR L17 OR L20
L22            3 DUP REM L21 (0 DUPLICATES REMOVED)

=> d que l22
L1             5 SEA FILE=REGISTRY CTGGATCCTTGCCCCGCCCCTTGAAT|TGGATCCTTGCCCCGCCC
                 CTTGAATT|GGATCCTTGCCCCGCCCCTTGAATTC|GATCCTTGCCCCGCCCCTTGAATTCC|
                 ATCCTTGCCCCGCCCCTTGAATTCCC/SQSN
L3           128 SEA FILE=HCAPLUS REICH N?/AU
L4           579 SEA FILE=HCAPLUS FLYNN J?/AU
L5           699 SEA FILE=HCAPLUS L3 OR L4
L6             3 SEA FILE=HCAPLUS L1
L9             3 SEA FILE=HCAPLUS L5 AND L6
L11            3 SEA FILE=HCAPLUS L6 AND (METHYLTRANSFERASE# OR METHYL(A)TRANSFE
                 RASE#)
L16          635 SEA FILE=HCAPLUS METHYL?(3A)DINUCLEOTIDE#
L17            1 SEA FILE=HCAPLUS L16 AND L6
L19           42 SEA FILE=HCAPLUS L16 (L) (METHYLTRANSFERASE# OR METHYL(A)TRANSF
                 ERASE#)
L20            1 SEA FILE=HCAPLUS L19 AND L6
L21            3 SEA FILE=HCAPLUS L6 OR L9 OR L11 OR L17 OR L20
L22            3 DUP REM L21 (0 DUPLICATES REMOVED)


=> d ibib abs l22 1-3

L22  ANSWER 1 OF 3  HCAPLUS  COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER:         1999:189269  HCAPLUS
DOCUMENT NUMBER:          130:232472
TITLE:                    Modulators of DNA cytosine-5 methyltransferase
```

Search completed by David Schreiber 308-4292

and methods for use thereof
INVENTOR(S): **Reich, Norbert O.; Flynn, James**
PATENT ASSIGNEE(S): The Regents of the University of California, USA
SOURCE: PCT Int. Appl., 114 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9912027	A1	19990311	WO 1998-US12351	19980612
W: CA, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2300354	AA	19990311	CA 1998-2300354	19980612
EP 1018003	A1	20000712	EP 1998-930207	19980612
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001514862	T2	20010918	JP 2000-508978	19980612
US 2003114402	A1	20030619	US 2001-10476	20011207

PRIORITY APPLN. INFO.: US 1997-57411P P 19970829
WO 1998-US12351 W 19980612
US 2000-485071 A3 20000203

AB A synthetic oligonucleotide comprising a C-5 methylcytosine and which recognizes and binds an allosteric site on DNA **methyltransferase** thereby inhibiting DNA **methyltransferase** activity is disclosed. Also disclosed is a compn. comprising a synthetic oligonucleotide of the invention. The compn. is useful for inhibiting DNA **methyltransferase** activity, thereby inhibiting the methylation of DNA. The compn. can be a pharmaceutical compn. useful for treating disorders assocd. with methylation defects, such as cancer and certain developmental disorders. Also disclosed is a method of inhibiting methylation of DNA. The method involves contacting a DNA cytosine-5 **methyltransferase** with a synthetic oligonucleotide of the invention in the presence of the DNA, thereby resulting in an enzyme/synthetic oligonucleotide complex. The presence of the complex prevents catalysis, thereby inhibiting DNA **methyltransferase** activity. Also disclosed is a method of treating a disorder of cell proliferation or development by administering to a subject a synthetic oligonucleotide of the invention. The inhibition of DNA **methyltransferase** prevents the methylation of DNA thereby treating the disorder of cell proliferation or development.

REFERENCE COUNT: 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1998:387706 HCAPLUS
DOCUMENT NUMBER: 129:158334
TITLE: DNA binding discrimination of the murine DNA cytosine-C5 **methyltransferase**
AUTHOR(S): **Flynn, James**; Azzam, Ramzi; **Reich, Norbert**
CORPORATE SOURCE: Department of Chemistry and Program in Biochemistry and Molecular Biology, University of California, Santa Barbara, CA, 93106, USA
SOURCE: Journal of Molecular Biology (1998), 279(1), 101-116
CODEN: JMOBAK; ISSN: 0022-2836

Search completed by David Schreiber 308-4292

Lewis 09/485,071

PUBLISHER: Academic Press Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Mammalian DNA cytosine-C5 **methyltransferase** modifies the CpG **dinucleotide** in the context of many different genomic sequences. A rigorous DNA binding assay was developed for the murine enzyme and used to define how sequences flanking the CpG dinucleotide affect the stability of the enzyme:DNA complex. Oligonucleotides contg. a single CpG site form reversible 1:1 complexes with the enzyme that are sequence-specific. A guanine/cytosine-rich 30 base-pair sequence, a mimic of the GC-box cis-element, bound threefold more tightly than an adenine/thymine-rich sequence, a mimic of the cAMP responsive element. However, the binding discrimination between hemi- and unmethylated forms of these DNA substrates was small, as we previously obsd. at the KmDNA level (Biochem., 35, 7308-7315 (1996)). Single-stranded substrates are bound much more weakly than double-stranded DNA forms. An in vitro screening method was used to select for CpG flanking sequence preferences of the DNA **methyltransferase** from a large, divergent population of DNA substrates. After five iterative rounds of increasing selective pressure, guanosine/cytosine-rich sequences were abundant and contributed to binding stabilization for at least 12 base-pairs on either side of a central CpG. Our results suggest a read-out of sequence-dependent conformational features, such as helical flexibility, minor groove dimensions and crit. phosphate orientation and mobility, rather than interactions with specific bases over the course of two complete helical turns. Thus, both studies reveal a preference for guanosine/cytosine deoxynucleotides flanking the cognate CpG. The enzyme specificity for similar sequences in the genome may contribute to the in vivo functions of this vital enzyme.

REFERENCE COUNT: 78 THERE ARE 78 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1996:304202 HCAPLUS
DOCUMENT NUMBER: 125:4242
TITLE: Murine DNA Cytosine-C5 **Methyltransferase**: Pre-Steady- and Steady-State Kinetic Analysis with Regulatory DNA Sequences
AUTHOR(S): **Flynn, James**; Glickman, J. Fraser; **Reich, Norbert O.**
CORPORATE SOURCE: Department of Chemistry, University of California, Santa Barbara, CA, 93106, USA
SOURCE: Biochemistry (1996), 35(23), 7308-7315
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We present the first description of KmDNA, KdDNA, kcat, and kmethylation for a mammalian DNA **methyltransferase**. Homogeneous, 190 000 Mr DNA (cytosine-5-)-**methyltransferase** isolated from mouse erythroleukemia cells has turnover consts. of 0.15-0.59 h-1 with single-stranded and unmethylated double-stranded oligonucleotides contg. a single CpG dinucleotide. These substrates were designed to mimic DNA transcriptional cis elements previously reported to have cytosine C-5-methylated regulation. The rate-limiting step for these substrates is the methylation step itself. In contrast, hemimethylated double-stranded substrates show burst kinetics, consistent with a rapid methylation event (3 h-1) followed by a slower step which dets. steady-state kcat. Hemimethylated and unmethylated double-stranded DNA shows similar binding affinities; these results reveal the mol. basis for the enzyme's

Search completed by David Schreiber 308-4292

preference for hemimethylated DNA to be the Me transfer step. Substrates with multiple recognition sites do not show burst kinetics and have turnover rate consts. of 6 h-1. Catalytic turnover for the mammalian enzyme is thus approx. 20-fold slower than that for the related bacterial enzymes. Our combined results show quant. that one enzyme is certainly capable of both maintenance and de novo methylation and that maintenance of the genomic methylation pattern is preferred over the de novo establishment of new patterns. Direct comparison of the mammalian enzyme with the bacterial DNA cytosine-C5 **methyltransferase**, M.SssI, indicates dramatic differences in preferences for single-stranded, double-stranded, and hemimethylated double-stranded substrates. Moreover, the specificity hierarchy shown for the M.SssI is derived from very different changes in Km and catalysis than those obsd. for the mammalian DCMTase. These results demonstrate that the M.SssI, and perhaps other DNA cytosine **methyltransferases** from bacteria, is functionally dissimilar to the mammalian enzyme.